Litton BIONETICS

MUTAGENICITY EVALUATION OF FDA 75-87 PYRIDOXINE HYDROCHLORIDE

FINAL REPORT

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Final Report-Mutagenic Evaluation of FDA 75-87 (Pyridoxine Hydrochloride) 9/77

MUTAGENICITY EVALUATION

OF

FDA 75-87

PYRIDOXINE HYDROCHLORIDE

FINAL REPORT

SUBMITTED TO

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-87, Pyridoxine hydrochloride, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-87, Pyridoxine hydrochloride

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: December 29, 1976

2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-87, Pyridoxine hydrochloride

2. Test solvent: * Saline

3. Solubility of the test compound under treatment conditions: Soluble

4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: April 4, 1977

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.00775	01.25
1/2 50% Survival	0.01550	02.50
50% Survival	0.03100	05.00

*The concentration of solvent was equal to the highest volume of test material added.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000058560

B. TEST DATE: MAY 18, 1977

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	28	21	22	35	17	16	34	27	148	143
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.03100 %	---	---	26	26	12	16	17	10	36	24	145	164
	0.01550 %	---	---	22	31	12	18	10	10	32	35	155	140
	0.00775 %	---	---	23	25	19	14	12	15	21	30	148	139
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	30	31	22	23	19	10	37	32	222	195
		RAT	LIVER	26	37	20	18	19	17	39	40	147	182
		MONKEY	LIVER	18	15	17	31	23	21	36	37	192	133
POSITIVE CONTROL***		MOUSE	LIVER	502	490	260	256	874	911	>1000	>1000	624	889
		RAT	LIVER	274	374	241	149	938	732	>1000	>1000	>1000	>1000
		MONKEY	LIVER	370	215	173	160	738	901	>1000	937	>1000	>1000
TEST	0.03100 %	MOUSE	LIVER	28	27	15	22	13	18	26	42	182	169
	0.01550 %	MOUSE	LIVER	14	20	14	13	13	9	24	39	143	175
	0.00775 %	MOUSE	LIVER	25	20	12	20	17	10	38	35	157	178
	0.03100 %	RAT	LIVER	35	21	19	15	10	17	36	31	132	139
	0.01550 %	RAT	LIVER	18	14	15	13	10	12	21	26	132	116
	0.00775 %	RAT	LIVER	19	18	18	17	18	19	32	36	141	124
	0.03100 %	MONKEY	LIVER	25	22	17	13	21	13	38	45	153	147
	0.01550 %	MONKEY	LIVER	23	27	14	11	13	11	25	32	140	157
	0.00775 %	MONKEY	LIVER	20	20	15	14	11	16	39	27	161	145

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

**	TA-1535	MNNG	2 UG/PLATE	***	TA-1535	ANTH	100 UG/PLATE
	TA-1537	QM	20 UG/PLATE		TA-1537	ANQ	100 UG/PLATE
	TA-1538	NF	100 UG/PLATE		TA-1538	AAF	100 UG/PLATE
	TA-98	NF	100 UG/PLATE		TA-98	AAF	100 UG/PLATE
	TA-100	MNNG	2 UG/PLATE		TA-100	ANTH	100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

NONACTIVATION COMPOUND 000058560

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		85.71	1.45	11.59	5.46	13.83	19.91	2.64	CONTROLS
NAP		900.49	685.45	235.32	163.30	71.70	109.36	78.06	
NA1		85.38	2.23	3.17	3.65	7.48	19.27	5.25	TEST DATA
NA2		65.90	3.10	12.90	4.01	11.89	15.52	5.41	
NA3		71.07	3.66	6.37	2.86	8.84	15.44	5.92	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES ICRFLO/MOUSE COMPOUND 000058560

TEST	ORG	TA100 HIS FX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	84.43	7.09	5.62	9.43	9.46	6.93	15.63	NEGATIVE CONTROLS
ACT	A-C	55.84	4.18	5.48	8.48	8.79	6.90	22.67	
ACT	ALI	57.41	5.86	8.83	5.51	21.48	10.38	8.14	
ACT	ALU	58.69	8.09	8.92	7.42	14.70	5.15	15.00	
ACT	PLI	182.07	78.46	97.15	149.24	87.28	53.36	91.82	POSITIVE CONTROLS
ACT	PLU	91.94	9.12	11.54	76.16	74.60	19.04	17.34	
ACT	LI1	101.79	4.17	6.82	7.19	25.82	17.36	11.33	TEST COMPOUND
ACT	LI2	84.94	4.73	5.48	5.16	23.44	9.90	10.70	
ACT	LI3	85.94	5.90	5.74	6.82	25.12	13.61	8.04	
ACT	LU1	102.71	6.81	9.33	6.26	16.42	7.15	12.86	
ACT	LU2	65.39	8.64	13.81	6.04	18.35	6.52	7.95	
ACT	LU3	81.55	3.89	10.30	7.81	18.04	9.60	6.76	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES SPRDAW/RAT COMPOUND 000058560

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	20.79	6.55	31.96	10.56	13.11	15.60	10.18	NEGATIVE CONTROLS
ACT	A-C	18.64	40.87	3.14	5.06	15.56	12.85	7.44	
ACT	ALI	83.48	12.32	12.17	10.82	36.48	15.01	10.34	
ACT	ALU	64.68	4.05	6.98	10.33	17.83	15.67	10.27	
ACT	PLI	247.96	196.42	55.58	88.95	297.10	110.32	71.61	POSITIVE CONTROLS
ACT	PLU	52.63	7.32	17.21	160.05	124.87	17.35	7.69	
ACT	LI1	34.36	1.59	3.78	9.49	18.89	15.07	13.81	TEST COMPOUND
ACT	LI2	54.55	1.77	1.35	6.35	36.59	10.78	11.77	
ACT	LI3	42.75	2.58	2.08	17.75	30.71	14.48	11.62	
ACT	LU1	55.97	6.60	7.11	8.46	14.99	20.78	13.11	
ACT	LU2	60.71	4.59	12.78	9.21	17.33	24.77	14.96	
ACT	LU3	53.59	4.38	6.03	13.22	17.08	15.63	13.69	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES RHESUS/MONKEY COMPOUND 000058560

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	87.84	5.64	21.88	13.86	7.51	21.98	10.90	NEGATIVE CONTROLS
ACT	A-C	64.54	2.96	1.25	11.47	8.19	8.04	7.30	
ACT	ALI	80.09	5.54	4.98	12.37	14.55	16.25	6.21	
ACT	ALU	70.23	3.78	7.32	10.10	10.45	20.74	7.73	
ACT	PLI	284.60	56.90	37.74	154.01	201.65	68.25	54.23	POSITIVE CONTROLS
ACT	PLU	69.20	4.38	13.64	7.74	8.22	20.46	7.34	
ACT	LI1	77.67	5.82	8.40	11.35	15.38	4.67	2.66	TEST COMPOUND
ACT	LI2	80.31	5.23	6.36	7.38	13.60	8.65	3.90	
ACT	LI3	83.21	3.00	4.90	7.31	11.21	8.64	2.78	
ACT	LU1	83.04	5.45	5.62	6.36	9.81	5.53	2.50	
ACT	LU2	89.71	6.37	4.76	6.56	7.26	5.84	2.31	
ACT	LU3	98.75	4.05	3.98	6.65	9.08	8.73	3.06	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-87, Pyridoxine hydrochloride, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-87, Pyridoxine hydrochloride, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

D. M. Brusick 7.28.77

David J. Brusick, Ph.D. Date
Director
Department of Molecular Toxicology

Reviewed by:

Robert J. Weir, Ph.D. Date
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagnes to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

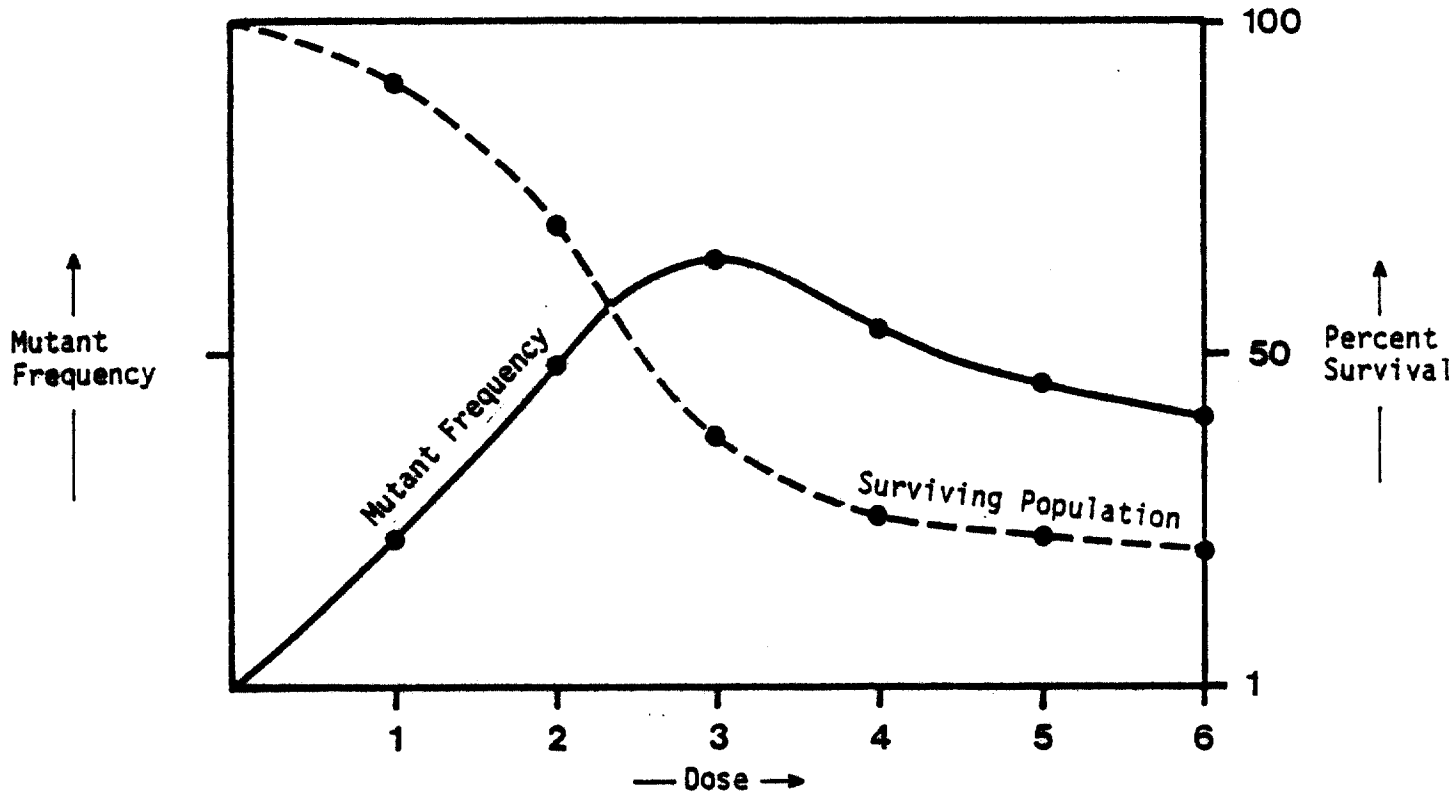
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710203 DETECTOR TA100 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0252	0216	85.71	0
	NAP		EMS 0.066%	0616	5547	900.49	0
000058560	NA1		0031-3 PCT.	0650	0555	85.38	0
000058560	NA2		0155-4 PCT.	0780	0514	65.90	0
000058560	NA3		0775-5 PCT.	0726	0516	71.07	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710201 DETECTOR TA1535 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	1310	0019	1.45	0
	NAP		EMS 0.2%	0852	5840	685.45	0
000058560	NA1		0031-3 PCT.	0851	0019	2.23	0
000058560	NA2		0155-4 PCT.	0969	0030	3.10	0
000058560	NA3		0775-5 PCT.	1066	0039	3.66	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710101 DETECTOR TA1537 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0466	0054	11.59	0
	NAP		QM 13 UG/ML	0235	0553	235.32	0
000058560	NA1		0031-3 PCT.	1701	0054	3.17	0
000058560	NA2		0155-4 PCT.	0589	0076	12.90	0
000058560	NA3		0775-5 PCT.	2088	0133	6.37	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705306 DETECTOR TA1538 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0403	0022	5.46	0
	NAP		NF 667 UG/ML	0376	0614	163.30	0
000058560	NA1		0031-3 PCT.	0521	0019	3.65	0
000058560	NA2		0155-4 PCT.	0449	0018	4.01	0
000058560	NA3		0775-5 PCT.	0454	0013	2.86	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710202 DETECTOR TA98 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0962	0133	13.83	0
	NAP		NF 667 UG/ML	0834	0598	71.70	0
000058560	NA1		0031-3 PCT.	1377	0103	7.48	0
000058560	NA2		0155-4 PCT.	1396	0166	11.89	0
000058560	NA3		0775-5 PCT.	1324	0117	8.84	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710902 DETECTOR 0000D4 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1175	0234	0031	19.91	2.64	1
	NAP		EMS 1.0 %	1463	1600	1142	109.36	78.06	0
000058560	NA1		0005-0 PCT.	1334	0257	0070	19.27	5.25	0
000058560	NA2		0025-1 PCT.	1276	0198	0069	15.52	5.41	0
000058560	NA3		0125-2 PCT.	1639	0253	0097	15.44	5.92	1

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710301 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0989	0835	84.43	0
	A-C		SOLVENT	0899	0502	55.84	0
	ALI		TISSUE	1545	0887	57.41	0
	ALU		TISSUE	1869	1097	58.69	0
	ACP	LI	DMN 90 UM/ML	1160	2112	182.07	0
	ACP	LU	DMN 90 UM/ML	0496	0456	91.94	0
000058560	ACT	LI1	0031-3 PCT.	0446	0454	101.79	0
000058560	ACT	LI2	0155-4 PCT.	0551	0468	84.94	0
000058560	ACT	LI3	0775-5 PCT.	0768	0660	85.94	0
000058560	ACT	LU1	0031-3 PCT.	0406	0417	102.71	0
000058560	ACT	LU2	0155-4 PCT.	0627	0410	65.39	0
000058560	ACT	LU3	0775-5 PCT.	0737	0601	81.55	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710303 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+8	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0649	0046	7.09	0
	A-C		SOLVENT	0431	0018	4.18	0
	ALI		TISSUE	0273	0016	5.86	0
	ALU		TISSUE	0346	0028	8.09	0
	ACP	LI	DMN 90 UM/ML	0687	0539	78.46	0
	ACP	LU	DMN 90 UM/ML	0570	0052	9.12	0
000058560	ACT	LI1	0003-3 PCT.	0623	0026	4.17	0
000058560	ACT	LI2	0155-4 PCT.	0550	0026	4.73	0
000058560	ACT	LI3	0775-5 PCT.	0458	0027	5.90	0
000058560	ACT	LU1	0003-3 PCT.	0499	0030	6.01	0
000058560	ACT	LU2	0155-4 PCT.	0521	0045	8.64	0
000058560	ACT	LU3	0775-5 PCT.	0591	0023	3.89	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710404 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1334	0075	5.62	0
	A-C		SOLVENT	1680	0092	5.48	0
	ALI		TISSUE	0804	0071	8.83	0
	ALU		TISSUE	1020	0091	8.92	0
	ACP	LI	AMQ 333 UG/ML	1297	1260	97.15	0
	ACP	LU	AMQ 333 UG/ML	1144	0132	11.54	0
000058560	ACT	LI1	0031-3 PCT.	1686	0115	6.82	0
000058560	ACT	LI2	0155-4 PCT.	1952	0107	5.48	0
000058560	ACT	LI3	0775-5 PCT.	1900	0109	5.74	0
000058560	ACT	LU1	0031-3 PCT.	0898	0083	9.33	0
000058560	ACT	LU2	0155-4 PCT.	0761	0099	13.01	0
000058560	ACT	LU3	0775-5 PCT.	1116	0115	10.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711201 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1219	0115	9.43	0
	A-C		SOLVENT	1226	0104	8.48	1
	ALI		TISSUE	1143	0063	5.51	0
	ALU		TISSUE	1145	0085	7.42	1
	ACP	LI	ANTH 67 UG/ML	1117	1667	149.24	1
	ACP	LU	ANTH 67 UG/ML	0302	0230	76.16	1
000058560	ACT	LI1	0031-3 PCT.	1391	0100	7.19	0
000058560	ACT	LI2	0155-4 PCT.	1144	0059	5.16	0
000058560	ACT	LI3	0775-5 PCT.	1108	0081	6.82	0
000058560	ACT	LU1	0031-3 PCT.	1310	0082	6.26	0
000058560	ACT	LU2	0155-4 PCT.	1307	0079	6.04	0
000058560	ACT	LU3	0775-5 PCT.	1268	0099	7.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710304 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1797	0170	9.46	0
	A-C		SOLVENT	1513	0133	8.79	0
	ALI		TISSUE	0810	0174	21.48	0
	ALU		TISSUE	1095	0161	14.70	0
	ACP	LI	ANTH 67 UG/ML	0629	0549	87.28	0
	ACP	LU	ANTH 67 UG/ML	1134	0846	74.60	0
000058560	ACT	LI1	0031-3 PCT.	0643	0166	25.82	0
000058560	ACT	LI2	0155-4 PCT.	0815	0191	23.44	0
000058560	ACT	LI3	0775-5 PCT.	0625	0157	25.12	0
000058560	ACT	LU1	0031-3 PCT.	0865	0142	16.42	0
000058560	ACT	LU2	0155-4 PCT.	0921	0169	18.35	0
000058560	ACT	LU3	0775-5 PCT.	1020	0184	18.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712902 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1184	0082	0185	6.93	15.63	0
	A-C		SOLVENT	1072	0074	0243	6.90	22.67	0
	ALI		TISSUE	1474	0153	0120	10.38	8.14	0
	ALU		TISSUE	1320	0068	0198	5.15	15.00	0
	ACP	LI	DMN 90 UM/ML	1222	0652	1122	53.36	91.82	0
	ACP	LU	DMN 90 UM/ML	1061	0202	0184	19.04	17.34	0
000058560	ACT	LI1	0031-3 PCT.	1659	0288	0188	17.36	11.33	0
000058560	ACT	LI2	0155-4 PCT.	1738	0172	0186	9.90	10.70	0
000058560	ACT	LI3	0775-5 PCT.	1991	0271	0160	13.61	8.04	0
000058560	ACT	LU1	0031-3 PCT.	1524	0109	0196	7.15	12.86	0
000058560	ACT	LU2	0155-4 PCT.	1686	0110	0134	6.52	7,95	0
000058560	ACT	LU3	0775-5 PCT.	1865	0179	0126	9.60	6.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711003 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0404	0084	20.79	0
	A-C		SOLVENT	0499	0093	18.64	0
	ALI		TISSUE	0230	0192	83.48	0
	ALU		TISSUE	0487	0315	64.68	0
	ACP	LI	DMN 90 UM/ML	0319	0791	247.96	0
	ACP	LU	DMN 90 UM/ML	0779	0410	52.63	0
000058560	ACT	LI1	0031-3 PCT.	0489	0168	34.36	0
000058560	ACT	LI2	0155-4 PCT.	0066	0036	54.55	0
000058560	ACT	LI3	0775-5 PCT.	0407	0174	42.75	0
000058560	ACT	LU1	0031-3 PCT.	0745	0417	55.97	0
000058560	ACT	LU2	0155-4 PCT.	0812	0493	60.71	0
000058560	ACT	LU3	0775-5 PCT.	0808	0433	53.59	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711080 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0229	0015	6.55	0
	A-C		SOLVENT	0208	0085	40.87	0
	ALI		TISSUE	0763	0094	12.32	0
	ALU		TISSUE	0469	0019	4.05	0
	ACP	LI	DMN 90 UM/ML	0447	0878	196.42	0
	ACP	LU	DMN 90 UM/ML	0287	0021	7.32	0
000058560	ACT	LI1	0031-3 PCT.	0692	0011	1.59	0
000058560	ACT	LI2	0155-4 PCT.	0734	0013	1.77	0
000058560	ACT	LI3	0775-5 PCT.	0581	0015	2.58	0
000058560	ACT	LU1	0031-3 PCT.	0500	0033	6.60	0
000058560	ACT	LU2	0155-4 PCT.	0392	0018	4.59	0
000058560	ACT	LU3	0775-5 PCT.	0297	0013	4.38	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710801 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0341	0109	31.96	0
	A-C		SOLVENT	0446	0014	3.14	0
	ALI		TISSUE	0871	0106	12.17	0
	ALU		TISSUE	0573	0040	6.98	0
	ACP	LI	AMQ 333 UG/ML	0851	0473	55.58	0
	ACP	LU	AMQ 333 UG/ML	0552	0095	17.21	0
000058560	ACT	LI1	0031-3 PCT.	0635	0024	3.78	0
000058560	ACT	LI2	0155-4 PCT.	0816	0011	1.35	0
000058560	ACT	LI3	0775-5 PCT.	0960	0020	2.08	0
000058560	ACT	LU1	0031-3 PCT.	0633	0045	7.11	0
000058560	ACT	LU2	0155-4 PCT.	0360	0046	12.78	0
000058560	ACT	LU3	0775-5 PCT.	0946	0057	6.03	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712501 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0956	0101	10.56	0
	A-C		SOLVENT	1244	0063	5.06	2
	ALI		TISSUE	0536	0058	10.82	0
	ALU		TISSUE	0552	0057	10.33	0
	ACP	LI	ANTH 67 UG/ML	0977	0869	88.95	0
	ACP	LU	ANTH 67 UG/ML	0443	0709	160.05	0
000058560	ACT	LI1	0031-3 PCT.	0917	0087	9.49	2
000058560	ACT	LI2	0155-4 PCT.	1149	0073	6.35	0
000058560	ACT	LI3	0775-5 PCT.	0614	0109	17.75	2
000058560	ACT	LU1	0031-3 PCT.	0721	0061	8.46	0
000058560	ACT	LU2	0155-4 PCT.	0630	0058	9.21	0
000058560	ACT	LU3	0775-5 PCT.	0658	0087	13.22	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 715101 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0915	0120	13.11	0
	A-C		SOLVENT	0405	0063	15.56	0
	ALI		TISSUE	0455	0166	36.48	0
	ALU		TISSUE	1127	0201	17.83	0
	ACP	LI	ANTH 67 UG/ML	0483	1435	297.10	0
	ACP	LU	ANTH 67 UG/ML	0961	1200	124.87	0
000058560	ACT	LI1	0031-3 PCT.	0884	0167	18.89	0
000058560	ACT	LI2	0155-4 PCT.	0533	0195	36.59	0
000058560	ACT	LI3	0775-5 PCT.	0495	0152	30.71	0
000058560	ACT	LU1	0031-3 PCT.	1161	0174	14.99	0
000058560	ACT	LU2	0155-4 PCT.	1160	0201	17.33	0
000058560	ACT	LU3	0775-5 PCT.	1089	0186	17.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712901 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1051	0164	0107	15.60	10.18	0
	A-C		SOLVENT	1533	0197	0114	12.85	7.44	0
	ALI		TISSUE	1306	0196	0135	15.01	10.34	0
	ALU		TISSUE	1091	0171	0112	15.67	10.27	0
	ACP	LI	DMN 90 UM/ML	1173	1294	0840	110.32	71.61	0
	ACP	LU	DMN 90 UM/ML	1118	0194	0086	17.35	7.69	0
000058560	ACT	LI1	0005-0 PCT.	1274	0192	0176	15.07	13.81	0
000058560	ACT	LI2	0025-1 PCT.	1317	0142	0155	10.78	11.77	0
000058560	ACT	LI3	0125-2 PCT.	1050	0152	0122	14.48	11.62	0
000058560	ACT	LU1	0005-0 PCT.	1083	0225	0142	20.78	13.11	0
000058560	ACT	LU2	0025-1 PCT.	0969	0240	0145	24.77	14.96	0
000058560	ACT	LU3	0125-2 PCT.	0979	0153	0134	15.63	13.69	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710401 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0839	0737	87.84	0
	A-C		SOLVENT	0863	0557	64.54	0
	ALI		TISSUE	0874	0700	80.09	0
	ALU		TISSUE	0823	0578	70.23	0
	ACP	LI	DMN 90 UM/ML	0617	1756	284.60	0
	ACP	LU	DMN 90 UM/ML	1013	0701	69.20	0
000058560	ACT	LI1	0031-3 PCT.	0918	0713	77.67	0
000058560	ACT	LI2	0155-4 PCT.	0904	0726	80.31	0
000058560	ACT	LI3	0775-5 PCT.	0840	0699	83.21	0
000058560	ACT	LU1	0031-3 PCT.	0802	0666	83.04	0
000058560	ACT	LU2	0155-4 PCT.	0816	0732	89.71	0
000058560	ACT	LU3	0775-5 PCT.	0639	0631	98.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710402 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0887	0050	5.64	0
	A-C		SOLVENT	0945	0028	2.96	0
	ALI		TISSUE	0903	0050	5.54	0
	ALU		TISSUE	0846	0032	3.78	0
	ACP	LI	DMN 90 UM/ML	1160	0660	56.90	0
	ACP	LU	DMN 90 UM/ML	1028	0045	4.38	0
000058560	ACT	LI1	0031-3 PCT.	1185	0069	5.82	0
000058560	ACT	LI2	0155-4 PCT.	1299	0068	5.23	0
000058560	ACT	LI3	0775-5 PCT.	1368	0041	3.00	0
000058560	ACT	LU1	0031-3 PCT.	1138	0062	5.45	0
000058560	ACT	LU2	0155-4 PCT.	1161	0074	6.37	0
000058560	ACT	LU3	0775-5 PCT.	1161	0047	4.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710901 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0352	0077	21.88	0
	A-C		SOLVENT	0641	0008	1.25	0
	ALI		TISSUE	0623	0031	4.98	0
	ALU		TISSUE	1229	0090	7.32	0
	ACP	LI	AMQ 333 UG/ML	1134	0428	37.74	0
	ACP	LU	AMQ 333 UG/ML	1158	0158	13.64	0
000058560	ACT	LI1	0031-3 PCT.	0405	0034	8.40	0
000058560	ACT	LI2	0155-4 PCT.	0550	0035	6.36	0
000058560	ACT	LI3	0775-5 PCT.	0816	0040	4.90	0
000058560	ACT	LU1	0031-3 PCT.	0747	0042	5.62	0
000058560	ACT	LU2	0155-4 PCT.	0882	0042	4.76	0
000058560	ACT	LU3	0775-5 PCT.	1181	0047	3.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712503 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0700	0097	13.86	2
	A-C		SOLVENT	0689	0079	11.47	2
	ALI		TISSUE	0590	0073	12.37	2
	ALU		TISSUE	0802	0081	10.10	2
	ACP	LI	ANTH 67 UG/ML	0798	1229	154.01	2
	ACP	LU	ANTH 67 UG/ML	1046	0081	7.74	2
000058560	ACT	LI1	0031-3 PCT.	0608	0069	11.35	2
000058560	ACT	LI2	0155-4 PCT.	0813	0060	7.38	2
000058560	ACT	LI3	0775-5 PCT.	0971	0071	7.31	2
000058560	ACT	LU1	0031-3 PCT.	0708	0045	6.36	2
000058560	ACT	LU2	0155-4 PCT.	0762	0050	6.56	2
000058560	ACT	LU3	0775-5 PCT.	0963	0064	6.65	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710403 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1852	0139	7.51	0
	A-C		SOLVENT	1660	0136	8.19	0
	ALI		TISSUE	0852	0124	14.55	0
	ALU		TISSUE	1167	0122	10.45	0
	ACP	LI	ANTH 67 UG/ML	1150	2319	201.65	0
	ACP	LU	ANTH 67 UG/ML	1789	0147	8.22	0
000058560	ACT	LI1	0031-3 PCT.	1248	0192	15.38	0
000058560	ACT	LI2	0155-4 PCT.	1191	0162	13.60	0
000058560	ACT	LI3	0755-5 PCT.	1543	0173	11.21	0
000058560	ACT	LU1	0031-3 PCT.	1641	0161	9.81	0
000058560	ACT	LU2	0155-4 PCT.	1846	0134	7.26	0
000058560	ACT	LU3	0755-5 PCT.	2169	0197	9.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711302 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1110	0244	0121	21.98	10.90	0
	A-C		SOLVENT	1343	0108	0098	8.04	7.30	0
	ALI		TISSUE	1385	0225	0086	16.25	6.21	0
	ALU		TISSUE	1268	0263	0098	20.74	7.73	0
	ACP	LI	DMN 90 UM/ML	1370	0935	0743	68.25	54.23	0
	ACP	LU	DMN 90 UM/ML	1212	0248	0089	20.46	7.34	1
000058560	ACT	LI1	0005-0 PCT.	1841	0086	0049	4.67	2.66	0
000058560	ACT	LI2	0025-1 PCT.	1745	0151	0068	8.65	3.90	0
000058560	ACT	LI3	0125-2 PCT.	1007	0087	0028	8.64	2.78	0
000058560	ACT	LU1	0005-0 PCT.	2605	0144	0065	5.53	2.50	0
000058560	ACT	LU2	0025-1 PCT.	1429	0072	0033	5.04	2.31	1
000058560	ACT	LU3	0125-2 PCT.	1569	0137	0048	8.73	3.06	0